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1: Mol Biochem Parasitol 1995 Dec;75(1):25-31

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Expression of mammalian cytokines by *Trypanosoma cruzi* indicate unique signal sequence requirements and processing.

La Flamme AC, Buckner FS, Swindle J, Ajioka J, Van Voorhis WC.

Department of Pathobiology, University of Washington, Seattle 98195, USA.

A vector based upon the calmodulin-ubiquitin 2.65 locus of *Trypanosoma cruzi* has enabled the expression and secretion of the murine cytokines interleukin-2 (IL-2) and gamma-interferon (gamma-IFN) by transfected *T. cruzi*. The *T. cruzi*-derived cytokines were bioactive and produced by both epimastigotes and mammalian form. The native coding sequence of IL-2 was sufficient to cause secretion of the protein. The gamma-IFN signal sequence had to be replaced by the IL-2 signal sequence (IL-2/gamma-IFN) to allow efficient secretion of gamma-IFN. The amino acid sequences at the N-termini of the secreted *T. cruzi*-derived cytokines were different from the expected murine secreted protein. The secreted IL-2 was cleaved six amino acids downstream from the murine signal sequence cleavage site, and the hybrid IL-2/gamma-IFN molecule was cleaved three amino acids downstream from the predicted signal cleavage site in the IL-2/gamma-IFN molecule. These apparent differences in signal peptide sequence requirements and cleavage sites most likely indicate that the signal sequence processing in trypanosomes is distinct from that of higher eukaryotes.

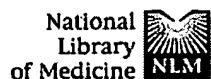
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1: Behring Inst Mitt 1991 Jul;89:12-22

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Modification of the cleavage activation of the influenza virus hemagglutinin by site-specific mutagenesis.

Garten W, Vey M, Ohuchi R, Ohuchi M, Klenk HD.

Institut fur Virologie, Philipps-Universitat Marburg, Germany.

Factors determining cleavability of influenza virus hemagglutinin which is activated by ubiquitous cellular endoproteases were analysed by carrying out site-directed mutagenesis on the cloned hemagglutinin genes of strains A/FPV/Rostock/34 (subtype H7) and A/Port Chalmers/1/73 (subtype H3). Substitutions at the cleavage site of the H7 hemagglutinin indicate that the tetrapeptide Arg-X-Lys/Arg-Arg is the minimal consensus sequence recognized by the ubiquitous proteases. The H3 hemagglutinin also became susceptible to these enzymes, when additional arginines were inserted at the cleavage site. Three arginines were sufficient, when the carbohydrate was removed, whereas four additional arginines are needed when this carbohydrate was present, indicating that the accessibility of the cleavage motif is important for the protease. The appropriate localization of the basic cleavage motif within the amino acid sequence and the spatial structure of the hemagglutinin precursor is an additional prerequisite for cleavage.

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1: Protein Expr Purif 1995 Oct;6(5):625-631

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Expression and functional characterization of *Escherichia coli* Nus and lambda Q as glutathione S-transferase fusion proteins.

Zhang Y, Hanna MM.

Department of Botany-Microbiology, University of Oklahoma, Norman 73019, US

The *Escherichia coli* transcription factor NusA and the bacteriophage lambda antiterminator Q proteins were expressed as inducible glutathione S-transferase (G fusion proteins. The fusion proteins were purified under nondenaturing conditions affinity chromatography on glutathione agarose. Thrombin cleavage of the glutathi agarose-bound fusion proteins yielded homogeneously pure NusAN+15 (5 mg/g ce and almost homogeneously pure QN+13 protein (0.7 mg/g cells), where N+x indic the presence of x additional amino acids at the N-terminus of the protein. The puri NusAN+15 exhibited the same activities as wildtype NusA in enhancement of transcriptional pausing, enhancement of termination at Rho-independent terminato and enhancement of Q-mediated antitermination in vitro. The QN+13 protein exhibited both anti-pausing and antitermination activities in Q-mediated transcripti antitermination. However, the antitermination activity of QN+13 was lost graduall during storage if the thrombin used for cleavage of the GST fusion protein was not removed. This was due to cleavage by thrombin after Arg22 within the Q protein itself, at a noncanonical thrombin cleavage site, so the truncated protein (QN+22) lacked the first 22 amino acids at the N-terminus of Q. The expression vectors described here can be used to rapidly produce large quantities of these proteins, and the truncated Q protein can be used to evaluate the requirement for the N-terminus Q in antitermination, anti-pausing, interactions with the DNA template (qut site), a interaction with RNA polymerase itself.

PMID: 8535155 [PubMed - indexed for MEDLINE]

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Structural features of a polypeptide carrier promoting secretion of beta-lactamase fusion protein in yeast.

Jamsa E, Holkeri H, Vihinen H, Wikstrom M, Simonen M, Walse B, Kalkkinen N, Paakkola J, Makarow M.

Institute of Biotechnology, University of Helsinki, Finland.

Escherichia coli beta-lactamase was secreted into the culture medium of *Saccharomyces cerevisiae* in biologically active form, when fused to the C-terminal of the hsp150 delta-carrier. The hsp150 delta-carrier is an N-terminal fragment of the yeast hsp150 protein, having a signal peptide and consisting mostly of a 19 amino acid peptide repeated 11 times in tandem. Here we expressed the hsp150 delta-carrier fragment alone in *S. cerevisiae*. Apparently due to a positional effect of the gene insertion, large amounts of the hsp150 delta-carrier were synthesized. About half of the de novo synthesized carrier molecules were secreted into the culture medium, the rest remaining mostly in the pre-Golgi compartment. The extensively O-glycosylated carrier fragment was purified from the culture medium under non-denaturing conditions. Circular dichroism spectroscopy showed that it had no regular secondary structure. Nuclear magnetic resonance spectroscopy showed that a non-glycosylated synthetic peptide, the consensus sequence of the repetitive 19 amino acid peptide, lacked secondary structure. The unstructured carrier polypeptide may facilitate protein folding and secretion of heterologous proteins attached to it.

PMID: 8585321 [PubMed - indexed for MEDLINE]

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